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Project 123-052

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FINAL REPORT

For period June 28, 1951 - June 28, 1954

Report prepared by Harry Sobotka, Ph.D.

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Title of Project: HEAT-STABLE PROTEINS AND ENZYMES

Objectives: Preparation and study of proteins and enzymes derived from thermophilic microorganisms.

SUMMARY OF RESULTS

a. Since start of project.

During the preceding periods we have developed synthetic media for the growth of a variety of thermophilic microorganisms of the genus Bacillus. These media consist of (1) a basic medium comprising a carbon source, a source of nitrogen, a mixture of trace elements kept for the greater part in the non-toxic ionized state by the addition of non-metabolizable chelating agents, and non-metabolizable pH-buffer. (2) To these were added certain amino acids and vitamins depending on the specific requirements of individual strains. By eliminating such poorly defined natural ingredients as liver, yeast, protein hydrolysates, etc., we were able to obtain media which kept clear at high temperatures, moreover, this was the only method by which reproducible growth could be obtained for these unique microorganisms. By controlling the medium in this way, we were enabled to grow these microorganisms with uniform enzyme content.

b. During current report period.

During the last six months we have grown selected strains of B. stearothermophilus and B. coagulans at 55°, 75°, and 80° C. in mass-cultures. Because of the rapid lysis of the bacilli at these temperatures, we can determine the viable growth by measuring optical density, which is proportional to growth. The actual optical densities surpass values of O.D. = 3 (equiv. to 1 1/2 - 2 g. dry bacterial substance per litre). The bacteria have been filtered off and were shown to contain ca. 75 % protein which was studied in regard to its chemical characteristics by paper electrophoresis, amino nitrogen according to Van Slyke, lipoid content and coagulation temperature.

Because of the rapid lysis of bacteria at these temperatures no ghosts are left in the medium, resulting in an optically clear supernatant which contains large quantities of thermostable proteins and enzymes which originate from living bacteria and from lysed bacteria.

These proteins are being compared with proteins made heat-stable by the addition of fatty acids and acetyl tryptophan. The thermophilic character of these proteins was proved by determination of their coagulation point using albumin as standard. We have also been able to show that the lipoids in the thermophilic proteins have a substantially higher iodine number.

PLANS FOR THE FUTURE

We expect to continue these studies with a view to (a) raising the upper temperature limit still further, in order to dissect their different metabolic pathways; (b) to detect the chemical features responsible for thermophily; (c) to compare the thermophilic enzymes with the thermophilic organisms in respect to the width of their temperature range; (d) we are utilizing some of these strains for the microbiological assay of folic acid, cobalamin and thioctic acid, for which they offer great advantages in regard to sensitivity, specificity and economy, particularly the non-requirement of sterilization.

REPORTS AND PUBLICATIONS

Herman Baker, Harry Sobotka, and Seymour H. Hutner, "Hot Bacteria", Office of Naval Research. In press.

Additional publications are in preparation.

Refer to preceding reports for previous publications.